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Airborne Pollen Calendar of Toluca City, Mexico

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Abstract: Allergic diseases are a global health problem; their prevalence has increased in recent decades. The presence of allergenic airborne pollen is one of the main triggers of this disorder. For this reason, the first pollen calendar of Toluca City was developed. Daily and bihourly airborne pollen samplings with a Hirst-type Burkard Trap were performed from August 2009 to December 2013. Annual Pollen Integral (APIn), Main Pollen Season (MPS) and Diurnal Pattern (DP) were determined. Relationships with meteorological parameters were investigated. Tree pollen grain presented higher concentrations, with Cupressaceae as the most abundant taxon (52.6%), followed by *Alnus* sp. (13.3%), Pinaceae (7.3%), *Fraxinus* sp. (6.0%) and *Quercus* sp. (2.0%), which presented a definite seasonality. Urticaceae (3.7%) was the most abundant herbaceous pollen taxon registered. The DP obtained showed that pollen grains of most taxa are frequently found after midday and afternoon. Regression models showed the influence of environmental variables on all taxa. This study will allow us the enhancement of preventive actions and improvement of the regional design of patient tests.

Keywords: airborne pollen; aeroallergens; aeropalynology; pollen allergy; pollen calendar; Cupressaceae; Toluca; Mexico

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1. Introduction

Allergy is a very common disease, affecting more than 20% of the population of most developed countries [1]. Allergic rhinitis is the most frequent disorder that affects approximately 25% of children and 40% of adults [2].

In Mexico City, the prevalence of allergic rhinitis is about 19.6% in the open population [3]; meanwhile, in the pediatric population, it is ranging from 11.3% to 15% [4]. It is considered that 40% of the population is sensitized to some allergen, mainly pollens and dust mites. There is a consistent association between sensitization to aeroallergens and allergic diseases such as asthma, rhinitis, and rhinoconjunctivitis [5].

Pollen grains are male gametophytes of seed-producing plants. They can be dispersed by air (anemophilous), insects (entomophilous) or both (ambiphilous). Allergenic pollen is primarily anemophilous [6–8]. A pollen grain is made up of proteins, lipids, polysaccharides and low molecular weight glycoproteins [9]; therefore, when they are kept in contact with the humid environment of the nasal and conjunctival mucosa, they spread rapidly because of their hydrophilic nature. Species-specific allergenic proteins are released by seasonal patterns [10]. The importance of carrying out pollen counts lies in the fact that the bigger the exposure, the greater the sensitization and severity of the symptoms of patients with pollinosis [11].

The temporal and spatial distribution of allergenic pollen types is crucial for the diagnosis and treatment of allergic diseases and their epidemiology. In order to detect probable triggers, it is important to guide a proper diagnostic testing to begin relevant therapy. Allergy sufferers and clinicians can utilize pollen calendars to consult and understand the distribution, timing, and concentration of various pollen species at specific places [12].

Despite the fact that the first record on atmospheric pollens in Mexico was carried out in 1949 for Salazar-Mallen [13], and several aeropollinic studies have been developed [14–25], only two pollen calendars have been elaborated, both for Mexico City [26,27]. Because pollen allergies display geographic variability, influenced by bioclimatic conditions and allergenic plants distribution [28], it is crucial to develop pollen calendars for each specific city in order to carry out a regional clinical management.

Toluca City is located within the fifth most populous metropolitan area in Mexico. It has a population of 910,608 inhabitants, which makes essential aerobiological surveillance. For this reason, Cid del Prado [29] carried out a preliminary study to determine the main airborne pollen types; however, a pollen calendar for this area has not been developed.

The objective of this work was the elaboration of the first pollen calendar of Toluca City in order to know the seasonality and diurnal pattern of main airborne pollen types and their relationship with meteorological variables.

2. Materials and Methods

2.1. Area of Study

Toluca City is located between latitude 18°59′ and 19°29′ north and longitude 99°32′ and 99°47′ west, at an average altitude of 2693 mamsl (Figure 1). The climatic types present in the city are subhumid–temperate, semi-cold and cold. The annual minimum average temperature is 6.6 °C, with an annual average temperature of 12.5 °C, and an annual maximum temperature of 28.8 °C. Total annual precipitation is 734.1 mm with an average relative humidity ranging from 52 to 77% [30]. Toluca City has protected natural areas, among which the Alameda Poniente Park stands out, with 12,729 ha of cedars and 10,069 ha of pines, as well as the Nevado de Toluca Flora and Fauna Conservation Area, which is populated by coniferous forest, oak and grassland, the Toluca Bicentennial Metropolitan Park where the Cupressaceae family predominates, and the Sierra Morelos State Park with a dominant forest cover of cedar, pine, eucalyptus and oaks. Therefore, arboreal vegetation is predominant [31].

Figure 1. Geographical location of Toluca City.

2.2. Pollen Monitoring

Continuous monitoring of pollen was carried out for 53 months (August 2009 to December 2013) with a Hirst-type Spore Trap (Burkard Manufacturing Co., Ltd., Rickmansworth, UK) at 10 m above ground level. It was placed in Medica Bosques Clinic (19°16′36.77″ N; 99°39′45.64″ W) at 2693 mamsl. This trap has a 14.2 mm inlet hole, and a drum in which airborne particles are impacted on a cellophane tape (Melinex) impregnated with silicone [32]. The drum is attached to a clock mechanism that moves 2 mm per hour, allowing the continuous and hourly sampling of particles in the air. It has a vane that keeps the air inlet hole in the direction of the prevailing wind, as well as a vacuum pump, which sucks 10 L of air per minute for seven days. The sampled tape was divided into sections equivalent to each sampling day (7 days—24 h). Each fragment was placed on a slide and mounted with glycerin jelly stained with fuchsine, and analyzed under the 40× objective Carl Zeiss light microscope. Hourly and daily impacted pollen types were counted, and data were analyzed following the recommendations of the REA (Spanish Aerobiology Network) [33]. For pollen identification, size, morphology and ornamentation were considered. The data obtained was pollen grain per m^3 of air (pg/m^3).

2.3. Determination of the Annual Pollen Integral (APIn) and Mean Pollen Season (MPS)

Annual total counts of pollen were taken into account for establishing Annual Pollen Integral (APIn) [34]. To determine the Mean Pollen Season (MPS), the data regarding the days of the beginning and end of the period of pollination were obtained using the cumulative method at 95% as it was mentioned by Andersen [35]. The first day on which an accumulated pollen concentration was equal to or greater than 2.5% was considered to be the beginning of the MPS. The last day the accumulated pollen concentration was equal to or lower than 97.5% was considered the end of MPS [36,37].

2.4. Pollen Calendar

The pollen calendar was constructed following Spieksma's model [38], which transformed 10-day mean pollen grain concentrations (pollen grain/m^3 of air) into a series of classes according to Stix and Ferretti [39] representing the series in a pictogram as an average of the four studied years. Each month was divided into three parts. This pictogram only presents pollen types with a minimum 10-day average equal to or higher than 1 pollen grain/m^3 of air.

2.5. Pollen Diurnal Pattern (DP)

The Diurnal Pattern (DP) was determined by calculating the average concentration for each two hours from MPS. This pattern only considered dry days without rainfall, when the concentration of pollen grains for each day was equal to or more than the daily average as suggested by Fernandez et al. and Calderon et al. [26,40].

2.6. Record of Meteorological Variables

Data of meteorological parameters from the sampling period were obtained from the Mariano Barcena Meteorological Observatory from the Universidad Autónoma del Estado de México located in the center zone of Toluca City, at 1.3 km from Hirst-type Spore Trap. Variables such as the mean, maximum, and minimum temperature, as well as accumulated precipitation, relative humidity, main, maximum and direction of wind speed were analyzed and correlated with the concentration of pollen grain registered.

2.7. Statistical Analysis

To determine the environmental variables that influence the presence of different pollen types in the air, multiple regressions were performed using the natural logarithm of the pollen counts +1 (to alleviate the positive skew found in the pollen concentrations values) as the response variable and the environmental variables as the regressor variables. The month was used as a co-variable in the regression models. The effects of the environmental variables were considered statistically significant when $p < 0.05$.

A correlation matrix between the environmental variables was constructed and plotted. Only significant Pearson correlations ($p < 0.05$) are shown in circles (positive = blue, negative = red) without a cross over them.

The analyses were performed in R 4.3.1, using *stats*, *corrplot*, *plyr*, *dplyr* and *ggplot2* packages.

3. Results

3.1. Pollen Monitoring

In Toluca City, there were 56 airborne pollen types identified (Table 1). The highest percentage of pollen types collected were from trees: Cupressaceae (52.6%), *Alnus* sp. (13.3%), Pinaceae (7.3%) and *Fraxinus* sp. (6.0%) as well as an herb type Urticaceae (3.7%) and Poaceae (3.1%) (Figure 2).

Table 1. Percentages of airborne pollen types collected in Toluca City from August 2009 to December 2013.

Pollen Type	%	Pollen Type	%
Cupressaceae	52.6348	Cyperaceae	0.1090
Alnus sp.	13.3545	*Plantago* sp.	0.0854
Pinaceae	7.3238	Brassicaceae	0.0622
Fraxinus sp.	6.0882	*Fagus* sp.	0.0363
Urticaceae	3.7784	*Tilia* sp.	0.0313
Poaceae	3.1964	Thypaceae	0.0302
Populus sp.	2.0956	*Citrus* sp.	0.0283
Quercus sp.	2.0123	*Jacaranda* sp.	0.0218
Asteraceae	1.2849	Onagraceae	0.0217
Moraceae	1.2566	Apiaceae	0.0206
Casuarina sp.	0.9628	*Ulmus* sp.	0.0189
Myrtaceae	0.6985	Solanaceae	0.0175
Ambrosia sp.	0.6135	Begoniaceae	0.0104
Amaranthaceae	0.6033	*Acacia* sp.	0.0102
Rosaceae	0.5125	*Carya* sp.	0.0097
Schinus sp.	0.4860	*Acer* sp.	0.0063
Salix sp.	0.3760	Lamiaceae	0.0052
Artemisia sp.	0.3249	*Grevillea* sp.	0.0027
Buddleia sp.	0.2519	Liliaceae	0.0024
Ricinus sp.	0.2372	*Wigandia* sp.	0.0019
Juglans sp.	0.2063	*Prosopis* sp.	0.0014
Rumex sp.	0.2035	*Vitacea* sp.	0.0014
Ligustrum sp.	0.1961	*Corylus* sp.	0.0009
Ficus sp.	0.1857	Sapindaceae	0.0009
Celtis sp.	0.1756	Acantaceae	0.0005
Mimosa sp.	0.1573	*Alyssum* sp.	0.0005
Palmae sp.	0.1253	Brassicaceae	0.0005
Liquidambar sp.	0.1196	*Tamarix* sp.	0.0005

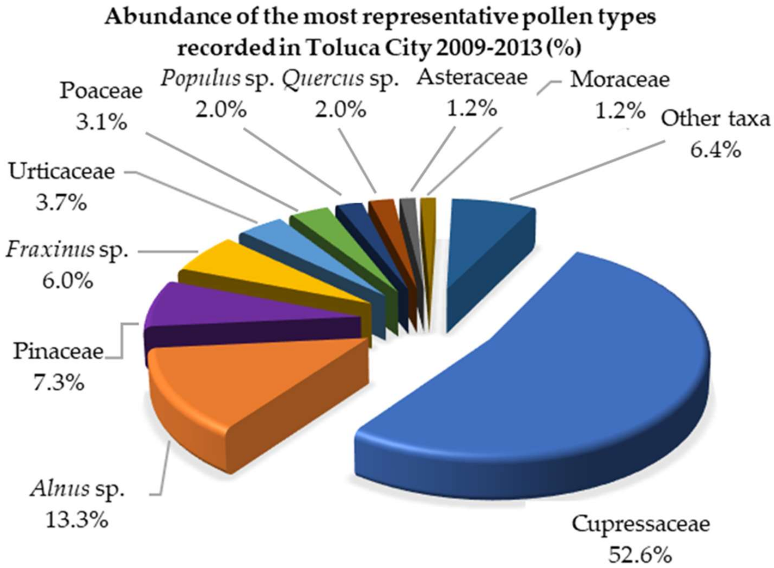

Figure 2. Abundance of pollen types recorded in Toluca City, 2009–2013.

3.2. Determination of the Annual Pollen Integral (APIn) and Mean Pollen Season (MPS)

The APIn for all pollen types was 51,890 for the period of 2009–2010; 34,449 for 2010–2011; 16,550 for 2011–2012 and 19,817 for 2012–2013. APIn values for main pollen types, dates for starting and ending of MPS, as well as maximum peaks dates are shown in Table 2.

Table 2. APIn, MPS and date of peak for main pollen types registered in Toluca City.

Pollen Type	Period	APIn	MPS Start Day	MPS End Day	Peak (pg/m^3)
Cupressaceae	2009–2010	28,876	23 September 2009	24 May 2010	28 January 2010 (787)
	2010–2011	17,872	19 August 2010	1 June 2011	16 January 2011 (686)
	2011–2012	8712	13 September 2011	18 May 2012	19 January 2012 (675)
	2012–2013	12,295	2 September 2012	6 July 2013	17 January 2013 (413)
Alnus sp.	2009–2010	8426	2 December 2009	30 March 2010	7 February 2010 (215)
	2010–2011	6018	11 December 2010	12 March 2011	13 January 2011 (207)
	2011–2012	2063	22 December 2011	23 March 2012	19 January 2012 (143)
	2012–2013	2022	29 November 2012	30 March 2013	15 January 2013 (54)
Pinaceae	2009–2010	3933	2 December 2009	14 May 2010	9 March 2010 (116)
	2010–2011	3380	7 December 2010	20 May 2011	28 February 2011 (228)
	2011–2012	1543	7 January 2012	4 June 2012	19 March 2012 (50)
	2012–2013	1071	21 December 2012	4 June 2013	27 February 2013 (35)
Fraxinus sp.	2009–2010	4142	17 November 2009	16 March 2010	21 January 2010 (157)
	2010–2011	2355	2 December 2010	14 March 2010	21 January 2011 (137)
	2011–2012	928	5 October 2011	31 March 2012	22 January 2012 (134)
	2012–2013	1148	16 November 2012	31 March 2013	2 February 2013 (59)
Urticaceae	2009–2010	1855	2 August 2009	22 July 2010	14 January 2010 (76)
	2010–2011	991	12 August 2010	26 July 2011	14 January 2011 (72)
	2011–2012	622	15 August 2011	30 July 2011	1 June 2012 (24)
	2012–2013	1502	12 August 2011	26 July 2013	3 June 2013 (55)

Table 2. *Cont.*

Pollen Type	Period	APIn	MPS Start Day	MPS End Day	Peak (pg/m^3)
Poaceae	2009–2010	1591	14 August 2009	17 July 2010	18 October 2009 (29)
	2010–2011	1107	10 August 2010	22 June 2011	12 October 2010 (19)
	2011–2012	354	9 August 2011	24 July 2012	25 September 2011 (7)
	2012–2013	637	12 August 2012	15 July 2013	11 September 2012 (17)
Populus sp.	2009–2010	1105	21 February 2010	19 April 2010	2 April 2010 (66)
	2010–2011	992	1 February 2011	10 May 2011	28 February 2011 (135)
	2011–2012	226	29 December 2011	11 April 2012	21 January 2012 (22)
	2012–2013	239	18 October 2012	24 March 2013	24 January 2013 (26)
Quercus sp.	2009–2010	501	9 February 2010	12 June 2010	25 March 2010 (63)
	2010–2011	854	3 March 2011	20 April 2011	23 March 2011 (69)
	2011–2012	1548	27 February 2012	31 May 2012	23 March 2012 (69)
	2012–2013	448	31 January 2013	24 June 2013	25 March 2013 (15)
Asteraceae	2009–2010	792	1 October 2009	8 June 2010	27 November 2009 (13)
	2010–2011	495	30 August 2010	21 June 2011	1 November 2010 (9)
	2011–2012	180	18 August 2011	8 July 2012	19 March 2012 (5)
	2012–2013	176	31 August 2012	22 May 2013	22 September 2012 (5)
Moraceae	2009–2010	669	9 October 2009	28 June 2010	7 February 2010 (18)
	2010–2011	385	12 August 2010	26 July 2011	5 March 2011 (21)
	2011–2012	374	19 September 2011	26 July 2012	5 March 2012 (21)
	2012–2013	279	14 August 2012	24 July 2013	24 November 2012 (18)

3.3. Pollen Calendar

A pollen calendar of mean pollen types was developed (Figure 3). Cupressaceae, the most abundant taxon, was observed all year round, reaching maximum values from December to February. Meanwhile, other tree pollen taxa presented a well-defined pollination season. In the case of *Pinaceae* sp., it was observed that pollination included the period from December to June with maximum levels in March. A similar pollination period for both *Alnus* sp. and *Fraxinus* sp. was observed from November to March with a peak in January. *Quercus* sp. was observed from February to June, *Populus* sp. was recorded from December to February. In contrast, the Moraceae tree pollen had a more extended pollination period (February–November), but lower pollen levels were found.

On the other hand, herbaceous pollen Urticaceae was found from December to September, with maximum levels in June. Asteraceae was recorded from September to October. Poaceae was found from July to January, with maximum values in September and October.

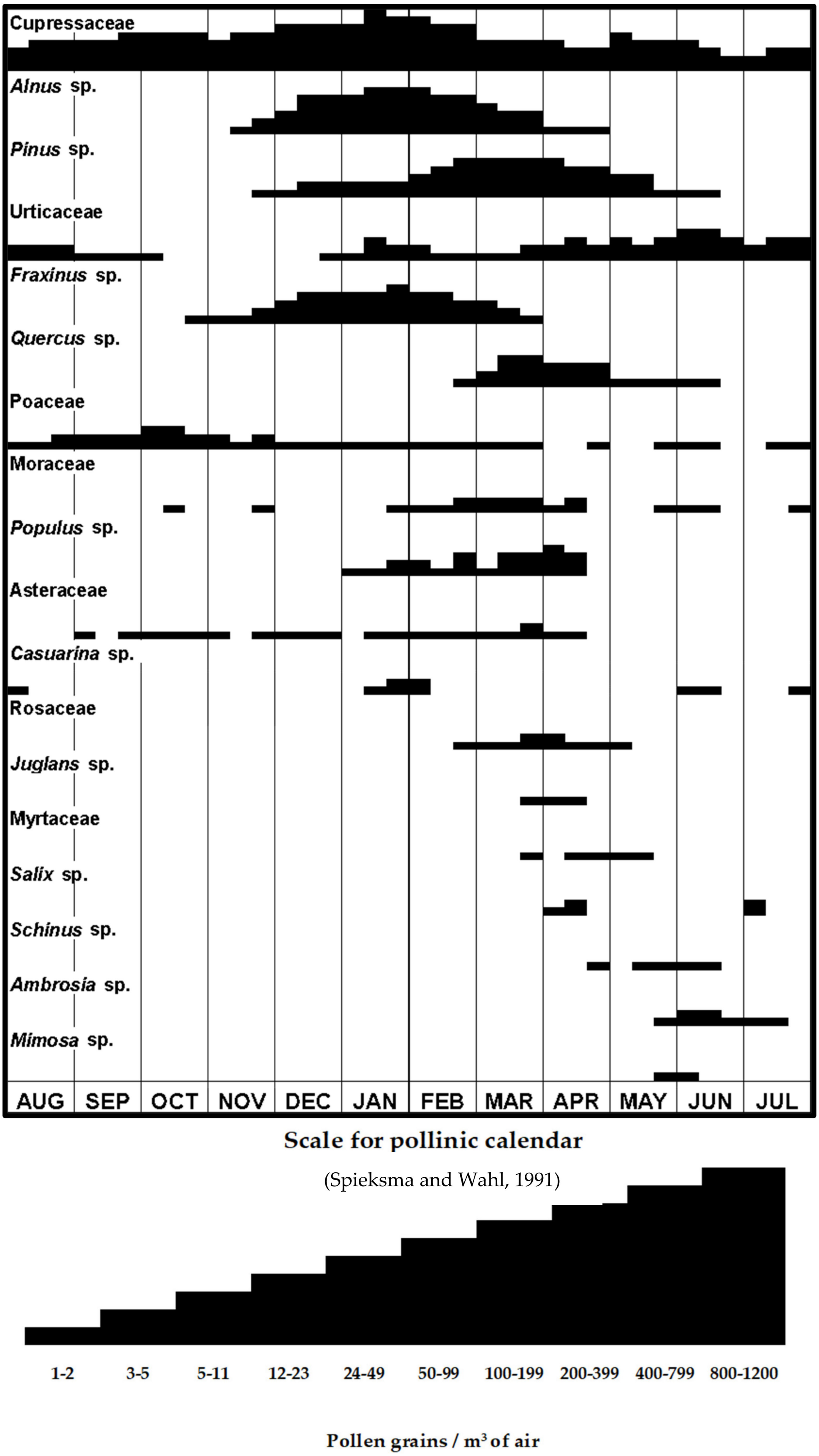

Figure 3. Pollen calendar of Toluca City, 2009–2013 [38].

3.4. Pollen Diurnal Pattern

Diurnal Pattern was obtained for the main pollen types. It can be seen that pollen grains are found frequently during midday and afternoon (Figure 4). Cupressaceae diurnal distribution had a well-defined pattern, reaching a maximum peak between 12:00 and 14:00 h; *Alnus* sp. showed a peak from 14:00 to 18:00 h. Pinaceae showed maximum values from 16:00 to 18:00 h. *Fraxinus* sp. had maximum values from 10:00 to 12:00. Urticaceae had a peak between 04:00 to 06:00. Poaceae pollen concentrations showed a peak from 10:00 to 16:00. *Populus* sp. reached a bimodal distribution, with the first peak from 08:00 to 10:00 and the second from 12:00 to 16:00 h. Meanwhile, *Quercus* sp. presented a bimodal distribution with two peaks, the first between 00:00 and 06:00 h and the second between 12:00 and 16:00 h. In contrast, Moraceae had a peak from 22:00 to 24:00 h. Asteraceae pollen grain concentration had two peaks, the first between 12:00 and 14:00 h and the second, most abundant, between 22:00 and 24:00 h (Figure 4).

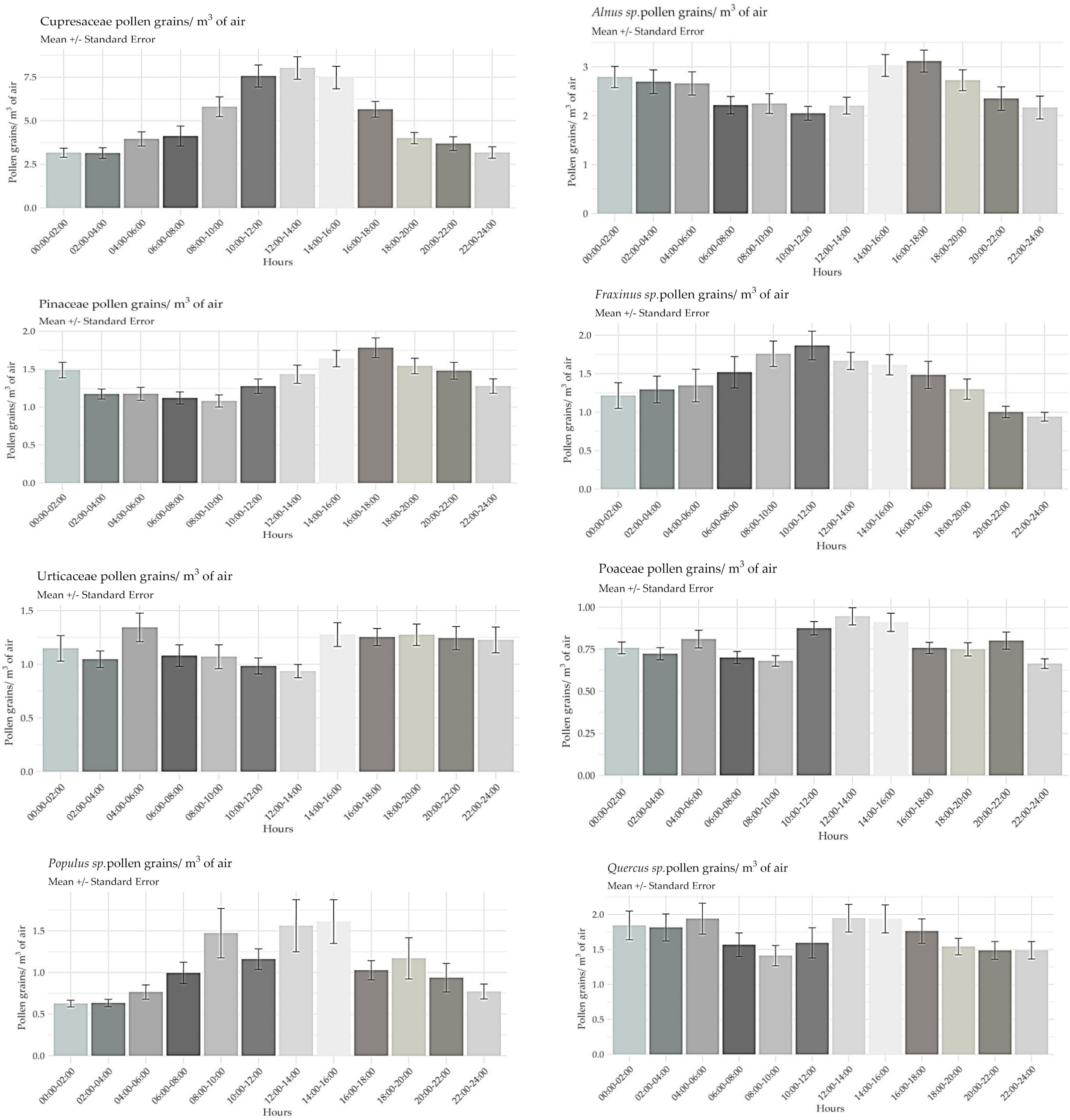

Figure 4. *Cont.*

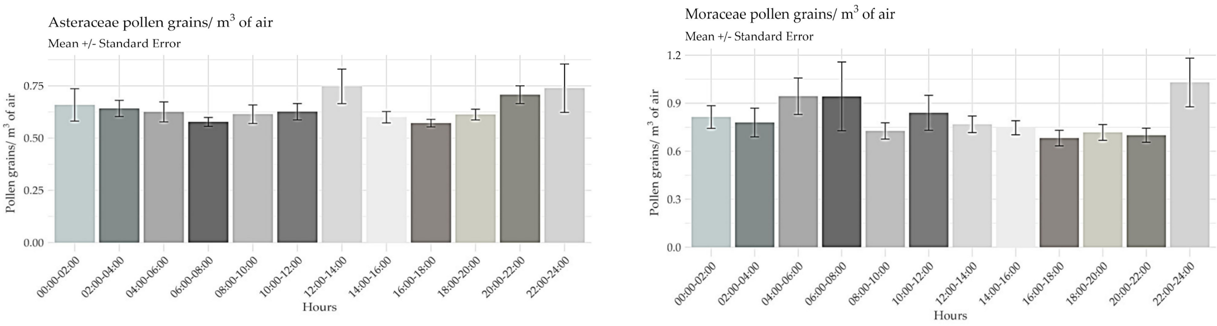

Figure 4. Diurnal Pattern for main pollen types in Toluca City.

3.5. Statistical Analysis of Meteorological Variables

Pearson's correlation matrix showed that minimum temperature, maximum temperature and mean temperature were positively and significantly correlated ($p < 0.05$). In addition, minimum temperature was positively and significantly correlated to accumulated precipitation (r = 0.72, $p < 0.05$). Accumulated precipitation was positively associated with relative humidity (r = 0.82, $p < 0.05$). Other relationships found such as the one between maximum temperature and relative humidity were weak and non-significant (r = −0.37, $p > 0.05$) (Figure 5).

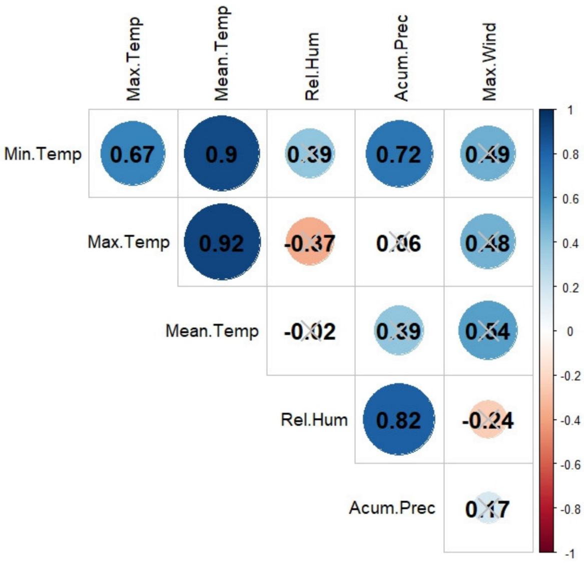

Figure 5. Pearson's correlation plot of environmental variables.

According to the multiple regression models built, the natural logarithm of pollen counts +1 of *Alnus* sp., Cupressaceae, *Fraxinus* sp. and *Populus* sp. reached their peaks during January, and significantly diminished during the following months. In the case of *Alnus* sp., it was negatively influenced by the minimum temperature ($\beta = -0.034835$, $p < 0.05$) and the relative humidity ($\beta = -0.011475$, $p < 0.001$). In the case of Cupressaceae, it was negatively influenced by accumulated precipitation ($\beta = -0.015580$, $p < 0.05$). Meanwhile, *Fraxinus* sp. was negatively influenced by minimum temperature ($\beta = -0.03147$, $p < 0.05$). In the case of *Populus* sp., it was negatively influenced by maximum wind ($\beta = -0.011270$, $p < 0.01$).

On the other hand, the natural logarithm of pollen concentrations +1 of Asteraceae significantly diminished in relation to January during the months of February and from May to August, and was negatively influenced by relative humidity ($\beta = -0.005909$, $p < 0.001$). In the case of Moraceae, it significantly increased in relation to January during March, May and June, and was negatively influenced by relative humidity ($\beta = -0.011658$,

$p < 0.001$). In the case of Poaceae, it significantly diminished in relation to January during the months of February, May and June, and significantly increased during September, October and November. It was negatively influenced by accumulated precipitation (β = −0.011886, $p < 0.01$) and positively influenced by mean temperature (β = 0.060977, $p < 0.001$). A two-factor effect was found on natural logarithm of pollen concentrations +1 of Urticaceae, which was negatively influenced by relative humidity (β = −0.008559, $p < 0.01$) and positively influenced by mean temperature (β = 0.059255, $p < 0.01$). In relation to January, it significantly increased from April to August and significantly decreased from August to December. Finally, mean temperature exerted a positive effect on the values of Pinaceae (β = 0.05602, $p < 0.001$) and *Quercus* sp. (β = 0.052875, $p < 0.001$). In relation to January, natural logarithm of pollen concentrations +1 of Pinaceae significantly increased from February to May and significantly decreased from June to December. In the case of *Quercus* sp., a significant increase was found from February to June, and a significant diminishment was found in September (Tables 3 and 4).

Table 3. Regression models for pollen grain concentrations and environmental variables.

Natural Logarithm ((Pollen Grains/m^3) + 1)	**Cupressaceae**	***Alnus* sp.**	**Pinaceae**	**Urticaceae**	***Fraxinus* sp.**
β_0 [1]	4.932006 ***	4.417650 ***	1.443387 ***	0.548883	2.66180 ***
Maximum Temperature					
Minimum Temperature		−0.034835 *			−0.03147 *
Mean Temperature				0.059255 **	
Relative Humidity		−0.011475 ***	−0.006106 **	−0.008559 **	
Accumulated Precipitation	−0.015580 *				
Maximum Wind					
February	−1.289357 ***	−1.119842 ***	0.812595***	−0.248057	−0.41023 ***
March	−2.726187 ***	−2.110270 ***	1.566601 ***	−0.038281	−1.37233 ***
April	−3.283674 ***	−3.048534 ***	1.157477 ***	0.416813 *	−1.88011 ***
May	−2.642208 ***	−3.127019 ***	0.623558 ***	0.529363 **	−2.11739 ***
June	−3.026183 ***	−3.130564 ***	−0.346016 **	1.425759 ***	−2.13691 ***
July	−2.813321 ***	−3.115850 ***	−0.736441 ***	1.006149 ***	−2.22179 ***
August	−2.982605 ***	−3.142952 ***	−0.902113 ***	0.597905 ***	−2.19974 ***
September	−2.009914 ***	−3.136628 ***	−0.933100 ***	0.136058	−2.16979 ***
October	−2.176874 ***	−3.207704 ***	−0.980717 ***	−0.433763 **	−2.09315 ***
November	−1.969916 ***	−2.643255 ***	−0.795771 ***	−0.534858 ***	−1.65974 ***
December	−2.230905 ***	−1.443778 ***	−0.813375 ***	−0.460733 *	−0.82703 ***
Adjusted R^2	0.4322	0.812	0.766	0.5097	0.6823

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. [1] β_0 is the natural logarithm of pollen concentration +1 during January when meteorological variables equal 0.

Table 4. Regression models for pollen grain concentrations and environmental variables.

Natural Logarithm ((Pollen Grains/m^3) + 1)	***Quercus* sp.**	**Poaceae**	**Moraceae**	***Populus* sp.**	**Asteraceae**
β_0 [1]	−0.540428 **	−0.126273	1.063619 ***	1.324396 ***	0.877043 ***
Maximum Temperature					
Minimum Temperature					
Mean Temperature	0.052875 ***	0.060977 ***			
Relative Humidity			−0.011658 ***		−0.005909 ***
Accumulated Precipitation		−0.011886 **			
Maximum Wind				−0.011270 **	
February	0.621898 ***	−0.249783 *	0.121445	−0.332803 ***	−0.225693 *
March	2.378970 ***	−0.146610	0.465995 ***	−0.876055 ***	−0.118467
April	2.091574 ***	−0.206340	0.037761	−0.998484 ***	−0.155658
May	0.561062 ***	−0.438997 ***	0.384426**	−1.098430 ***	−0.322176 ***
June	0.628190 ***	−0.485042 ***	0.293963*	−1.040607 ***	−0.357713 ***
July	0.001794	−0.250942	0.201948	−1.132223 ***	−0.277092 **
August	−0.213267	0.311679 **	0.120702	−1.094175 ***	−0.307733 ***
September	−0.214344 *	0.914706 ***	0.100338	−1.091790 ***	0.039722
October	−0.180243	0.669034 ***	0.056486	−1.052386 ***	0.060578
November	−0.113790	0.296006 **	−0.079498	−0.908857 ***	−0.098601
December	−0.003865	−0.245666	−0.200815	−0.756293 ***	−0.210484
Adjusted R^2	0.7539	0.4681	0.1396	0.3953	0.1427

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. [1] β_0 is the natural logarithm of pollen concentration +1 during January when meteorological variables equal 0.

Wind direction was analyzed monthly and annually for the determination of predominant wind. Southeast wind had more than 20% of occurrence in this period. Northeast wind presented values above 10% (Figure 6).

Figure 6. Predominant wind direction registered in Toluca City (%).

4. Discussion

The air of Toluca City was continuously monitored for 53 months to observe existing airborne pollen types in the area. The concentrations of total air pollen showed differences in the APIn between monitored years, the period of 2009–2010 displaying a considerably highest APIn (51,890 pg), which is coincident with the data observed in Mexico City for the same period [26,27], whereas the period from 2011 to 2012 was the lowest (16,550 pg).

These differences may be due to various causes already widely documented, such as interannual changes in meteorological conditions, changes in regional vegetation, differences in the flowering rhythm of each species, as well as the presence of biennial or triennial flowering plants [41].

The first pollen calendar of Toluca City was developed, which is the first calendar for this area and the third for Mexico. It was found that the most abundant taxon registered was the Cupressaceae family, which reached values of 52.6%, due to the abundance of trees of this family in the study area, which matches with the values obtained for this area (44.7%) during a previous study [29]. Reports in other countries also document Cupressaceae in the first place, as is the case of Ankara, Turkey [42], in contrast to what was reported for Mexico City and Monterrey, Mexico, where the genus *Fraxinus* sp. ranks first in abundance [25,27]. Cupressaceae pollen is the only pollen type that remained present throughout the year, with its maximum peak in January. It is an inaperturate spherical pollen ranging 20 to 35 µm in diameter, with thick intine and thin exine that can be shed after anthesis [43]; mainly after thunderstorms, they release submicron particles [44] that contain allergens, increasing the availability of airborne allergens which are capable of sensitizing an atopic individual and, in the following challenges, producing a reaction because of its high allergenicity [45]. It has been reported that an allergy activation threshold is at 50–60 pollen pg/m^3 air in the case of cypress [46].

The pollen of *Alnus* sp., a moderate allergen, was the second most abundant (13.3%), coinciding with the 12.0% abundance previously reported [29]. In contrast, it should be noted that the proportional percentage of the Pinaceae family was 7.3%, as opposed to the 13.8% previously reported, which could be due to a loss of Pinaceae in the protected zone of Nevado de Toluca as reported by Franco [47] which registered a rate of decrease of 39.7 ha/year in its area of distribution from 2001 to 2013. Therefore, this type of study can also be a bioindicator of the loss of tree populations. The importance of conservation of species as Pinaceae relies on this species preventing slopes from erosion because of the deep roots of these plants, as well as providing a cover composed of leaf litter, wood residues, bark, and cones. They form blankets that can reach eight centimeters in depth. This contribution of material supposes, on the one hand, the enrichment of organic matter and, on the other, an abundant fuel that puts its permanence at risk in the event of a fire attack. Furthermore, due to their vigorous root system, these trees serve as the most efficient mechanisms for the incorporation of rainwater into the soil [47].

Pollen grains of *Fraxinus* sp. had a proportion of 6.0% in contrast to reports for Mexico City where it ranks first in abundance and importance because its high allergenicity, in fact, was the second most prevalent allergen in a group of patients in three hospitals in Mexico City [26,27]. It was registered during winter with a peak in January, which coincides with pollination peaks of Cupresaceace and *Alnus* sp. About 80% of patients sensitized to *Fraxinus* sp. have a specific IgE to Fra e 1 [48].

The Urticaceae family presented 3.7% of abundance. This herbaceous species, which ranked fifth in our study, has also been reported in other investigations. It was also fifth in abundance in the state of Sonora, Mexico [49]. However, in other studies, it has been reported in the first position, such is the case of Augsburg, Germany [50]. Its allergenicity is variable, considered from low to high allergenicity, depending on the studied genus [51].

Poaceae pollen grains only had an abundance of 3.1% due to the fact that the vegetation is predominantly arboreal; however, because it is present throughout the year and has an allergy activation threshold of 3–5 pg/m^3 for hypersensitive patients [46], it can trigger allergic symptoms as reported by Calderón [26] in pediatric patients with allergic conjunctivitis and rhinitis.

Populus sp. presented an abundance of 2.0%. It is a genus belonging to the Salicaceae family; it is a tree mainly introduced in Mexico City and, to a lesser extent, in Toluca

City. Natural populations are only observed in the western zone of Mexico (Michoacán, Colima, Jalisco, Nayarit and Sinaloa) and in the north of Mexico [52]. Its allergenicity is moderate [53].

Quercus sp. was eighth in abundance, with a percentage of 2.0%, mainly because it is found in parks within the urban area, such as Alameda Poniente State Park and Sierra Morelos Park, as well as in forests outside the city, such as Nevado de Toluca and Sierra de las Cruces Natural Areas [54]. There are 23 species of *Quercus* documented for the State of Mexico, *Quercus crassipes* being the most abundant for Toluca [31]. Its allergenicity is reported as moderate–high [55].

Records of intraday variation patterns for Cupressaceae, *Fraxinus* sp., Poaceae, *Populus* sp., *Quercus* sp. and Asteraceae were collected during midday (between 10:00 and 16:00 h), which is associated with higher solar radiation as well as a drop of the relative humidity that favors convective air currents [56], while Pinaceae presented a gradual increase from 12:00, reaching its peak between 16:00 and 18:00 h, which may be associated with the fact that its populations are located at a greater distance from the point of sampling. Moraceae and Asteraceae presented a peak from 22:00 to 24:00, while Urticaceae had their peak during early morning before sunrise. This can be due to fact that pollen laden air rises to the upper atmosphere in convection currents during daytime, and pollen-bearing air descends at night as it cools, thereby increasing the concentrations of pollen at ground level as reported by Grewling et al. [56]. In addition, these taxa have small pollen grain, which allows them remaining at the atmosphere for a longer time.

Multiple regression models showed that temperature had a marked influence. A negative effect of temperature was observed for two pollen types with a marked seasonality, *Alnus* sp. and *Fraxinus* sp., which flower during the winter. The need to undergo a period of low temperatures ensures growth before flowering. This is the case for many biennial or perennial plants that need to undergo a period of cold to have optimal floral development, showing a marked dormancy period. In species with temperate or cold climates, this is an adaptive mechanism to avoid damage from inclement weather (avoiding freezing damage to cells). Their development is stopped almost in its entirety, allowing plants the entrance into a state of dormancy where the tissues are less sensitive to extreme temperatures [45,57]. In contrast, a positive effect of mean temperature was found for Urticaceae, *Quercus* sp. and Poaceae as reported for these taxa in Mexico City [26,58].

A negative effect of relative humidity or accumulated precipitation was observed for almost all pollen types (Cupressaceae, *Alnus* sp., Pinaceace, Urticaceae, Poaceae, Moraceae and Asteraceae) as previously reported in other studies [59]. Only a slight negative effect of maximum wind speed was found for *Populus* sp. The dominant wind direction for Toluca City was mainly from the southeast, which would mean transport of airborne pollen grains from forest areas such as Parque Ambiental Bicentenario.

Since this study is ten years old, these conditions have probably changed. High rates of deforestation have been reported in the last decade [47], the deforestation rate of the Nevado of Toluca has doubled due to a change in the protection category of the Protected Natural Area, and so did the changes in the meteorological conditions reported in the study area due to factors such as the increase in environmental contamination [26]. For this reason, it is planned to carry out a new monitoring from the year 2024 to determine the changes observed in the near future.

5. Conclusions

This research allows us to acquire knowledge about the existing pollen in the atmosphere of a city located at a high altitude and surrounded by arboreal vegetation. This information is very useful for local allergists, physicians and patients.

Airborne pollen monitoring is not only important for health issues, but it can also be used as a tool for ecological research of the effects of deforesting, environmental pollution, climate change and their impact on the phenology of the plants.

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